A Morphological and Genetic Analysis of Geographic Variation Among Oysters in the Gulf of Mexico

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INTRODUCTION

It has often been noted that oyster shells are highly variable in shape. This variation appears to correlate closely with changes in environmental variables. For instance, oysters growing singly on firm bottoms tend to develop round shells, whereas specimens living on soft bottoms usually have slender shells. Thus,variation in shell shape appears to provide information on the specific environment in which an oyster developed but is of questionable use in determining its geographic origin.

A variety of techniques have been applied to answer the question of whether geographic races of *Crassostrea virginica* (Gmelin, 1791) exist. Beginning with studies of differences in heat inducement of spawning (Stauber, 1950; Loosanoff & Nomejko, 1951), races of *C. virginica* have been based on chromatography of peptides (Hillman, 1964), serological tests (Li *et al.*, 1967), and electrophoresis of enzymes (Buroker *et al.*, 1979). These physiological and biochemical procedures provide data less influenced by environmental factors. However, there has been no study of the degree to which morphological variation reflects physiological or genetic differences. This study was undertaken to determine whether geographic races of oysters existed in the Gulf of Mexico on either side of the Mississippi River or along the Texas coast and to examine the correlation of morphological and biochemical variables in detecting geographic variation.

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METHODS AND MATERIALS

Collection Methods

Five collections of oysters were made at the following localities: 1) Biloxi Bay, Mississippi, 2) West Bay, Texas, near the Galveston airport, 3) Drum Bay, Texas, 4) Aransas Bay, Texas, and 5) lower Laguna Madre (near Port Isabel), Texas. All the collections were similar in that all five came from small shallow reefs in protected bays. The oysters were sampled without regard to size or location.

Electrophoretic Analyses

The collections were taken alive to the laboratory and frozen at −60° C. In processing, the oysters were partially thawed in water, removed from the shell and allowed to drain for 15 minutes before being weighed. The right valve was saved for later analysis. The adductor muscle and part of the digestive diverticula were then placed in separate test tubes with grinding buffer (0.1M Tris, 0.001M EDTA, 0.05M $CaCl_2$, 0.0005M NADP). The volume of the buffer was approximately one half that of the tissue. The tissue was then homogenized using a teflon tipped grinding bit and centrifuged for 20 minutes at 19000 rpm (46300g). The supernatant was then frozen until electrophoretic analysis could be carried out.

Starch gel electrophoresis was used to assess genetic variation (Selander *et al.*, 1971). Two buffer systems were used: 1) discontinuous Tris citrate (Poulik) gel; 2) lithium hydroxide gel.

Five enzyme stains were used (Selander *et al.*, 1971): Phosphoglucose Isomerase (PGI, EC 5.3.1.9), Phosphoglucomutase (PGM, EC 2.7.5.1) esterase with 4-methylumbel-

liferyl acetate as substrate (4MU EC 3.1.1), peptidase with l-leucyl-l-alanine as substrate (Leu-Ala, EC 3.4.1), Glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1). PGI, PGM, and GOT were analyzed on Poulik gels. 4MU was analyzed on LiOH gels. Leu-Ala resolved equally well on both gel types.

Each stain produced one scorable locus. At each locus the most common allozyme was designated M, those migrating faster (more anodally) F, and those slower (more cathodally) S. If more than one F or S existed, subscripts were consecutively assigned starting with the allozyme closest to M in mobility.

Morphological Analysis

Standard height and length measurements were made from the right valve for morphological comparisons (Galtsoff, 1964). The height was measured from the tip of the beak to the outer lip along a line touching the anterior edge of the muscle scar. The length was measured on a line perpendicular to the height line through the dorsal edge of the muscle scar. The ratio of length to height was computed as a measure of shell shape (Galtsoff, 1964). Also, all of the tissue was removed from the shell, blotted, and weighed to obtain a wet weight value.

Table 1

Allozyme frequencies.

Locus	Alleles	Biloxi Bay	West Bay	Drum Bay	Aransas Bay	Laguna Madre
PGI	F3	0.000	0.000	0.006	0.000	0.000
	F2	0.097	0.043	0.074	0.083	0.043
	F1	0.486	0.468	0.358	0.417	0.100
	M	0.389	0.473	0.534	0.431	0.557
	S1	0.028	0.016	0.006	0.056	0.186
	S1	0.000	0.000	0.023	0.014	0.017
	S3	0.000	0.000	0.000	0.000	0.043
		36[1]	94	88	36	35
PGM	F2	0.045	0.051	0.026	0.045	0.000
	F1	0.111	0.063	0.103	0.106	0.052
	M	0.722	0.659	0.718	0.667	0.741
	S1	0.125	0.204	0.154	0.167	0.155
	S2	0.000	0.023	0.000	0.015	0.052
		36	88	78	33	29
4MU	F1	0.000	0.000	0.006	0.000	0.000
	M	0.861	0.833	0.876	0.847	0.014
	S1	0.139	0.167	0.107	0.153	0.986
	S2	0.000	0.000	0.011	0.000	0.000
		36	93	89	36	36
Leu-Ala	F3	0.014	0.011	0.028	0.000	0.014
	F2	0.186	0.269	0.244	0.186	0.222
	F1	0.243	0.199	0.189	0.333	0.250
	M	0.343	0.393	0.422	0.375	0.375
	S1	0.214	0.124	0.100	0.097	0.111
	S2	0.000	0.005	0.017	0.014	0.028
		35	93	90	36	36
GOT	F3	0.000	0.000	0.000	0.000	0.056
	F2	0.016	0.005	0.006	0.000	0.000
	F1	0.500	0.484	0.478	0.403	0.847
	M	0.469	0.493	0.517	0.583	0.097
	S1	0.016	0.016	0.000	0.014	0.000
		32	92	90	36	36

[1]Number of individuals analyzed.

RESULTS

Allozyme frequencies for each locus are presented by collection in Table 1. The Laguna Madre collection is greatly different from all other collections. At the PGI locus, the F1 allozyme has a lower frequency in the Laguna Madre collection than in the others, with a concomitant increase in the S1 allozyme. The Laguna Madre collection is nearly monomorphic at the 4MU locus, and the common allozyme changes from the M to the S1 allozyme at Laguna Madre. In addition, the most frequent allele at the GOT locus is the F1 allozyme in the Laguna Madre sample, whereas the other samples have similar frequencies of the F1 and M allozymes.

Table 2

Genetic variation of oyster populations.

Population	Mean heterozygosity	Variance of heterozygosity
Biloxi Bay	0.513	0.035
West Bay	0.518	0.025
Drum Bay	0.494	0.033
Aransas Bay	0.521	0.029
Laguna Madre	0.418	0.081
Average (H_s)	0.493	

Goodness-of-fit of observed genotype frequencies to those expected from Hardy-Weinberg equilibrium was tested by a randomization computer program. The deviations that were found will be discussed in a subsequent paper (Groue and Lester, in preparation).

The amount of variation within each population was measured as heterozygosity. The heterozygosity for a locus (h) is defined as $1 - x_i^2$ where x_i is the frequency of the i^{th} allozyme, and average heterozygosity (H_s) is the mean of h over all loci examined. H_s is a good measure of diversity because it is sensitive to changes in both the number and frequencies of 'alleles' (Nei, 1975). Values for H_s as well as variances of h are presented in Table 2. The heterozygosity of the Laguna Madre sample is slightly below those of the other four, which are all similar.

Table 3

Genetic identities based on gene frequencies.

	Biloxi Bay	West Bay	Drum Bay	Aransas Bay
West Bay	0.992			
Drum Bay	0.987	0.993		
Aransas Bay	0.989	0.990	0.989	
Laguna Madre	0.641	0.659	0.640	0.628

The similarity between two populations is measured with the normalized genetic identity (I) (Nei, 1975). At the j locus this is defined as

$$I_j = \frac{\Sigma x_i\, y_i}{\sqrt{\Sigma x_i^2\ \Sigma y_i^2}}$$

where x and y represent the frequencies of the i^{th} allozyme in populations x and y, respectively. For all loci in a sample, the overall genetic identity of x and y is defined as

$$I = \frac{Jxy}{\sqrt{JxJy}}$$

where Jx, Jy and Jxy are the arithmetic means over all loci of Σx_i^2, Σy_i^2, and $\Sigma x_i y_i$, respectively.

The genetic identity (I) of each pair of populations is presented in Table 3. The West Bay, Drum Bay, Aransas Bay and Biloxi Bay collections are all similar with at least 98.7% of the mobility classes (alleles) being shared with other populations. Laguna Madre oysters share between 62% and 66% of the mobility classes (alleles) with other samples.

Table 4

Morphological measurements.

	Biloxi Bay	West Bay	Drum Bay	Aransas Bay	Laguna Madre
Height (cm)					
mean	7.36	6.40	6.85	8.46	5.99
S.D.	1.17	1.50	1.11	1.56	1.24
Length (cm)					
mean	5.27	4.99	4.37	4.54	3.16
S.D.	0.73	0.99	0.70	0.92	0.55
Weight (g)					
mean	13.60	11.92	9.94	12.68	6.10
S.D.	6.07	6.96	4.22	5.37	2.80
Length/height index					
mean	0.72	0.79	0.64	0.53	0.54
S.D.	0.10	0.14	0.11	0.09	0.14

Morphological Variation

The means and variances of the morphological measurements are presented in Table 4. In order to analyze differences in shape, the length/height ratio was calculated for each individual. Means and variances of this index are also presented in Table 4. The distributions of this index for each population were fitted to a normal distribution and none were found to deviate significantly ($p<0.25$).

A one-way analysis of variance was performed on this index and significant differences between populations were found ($F = 49.34$, degrees of freedom = 4 and 287, $p < 0.001$). A Student-Newman-Keuls (SNK) test (Sokal & Rohlf, 1969) found that only the Laguna Madre and Aransas Bay populations were not significantly different; all other combinations were significantly different.

DISCUSSION

Macrogeographic Differentiation

Previous studies have examined various forms of geographic variation in oysters and other marine bivalves and implied a genetic basis for that variation. Geographic variation was first demonstrated by Stauber (1950) and by Loosanoff & Nomejko (1951) who found differences in the spawning reactions of populations of oysters in response to water temperature. Spawning could be induced in northern oysters by a short exposure to warm water, whereas southern oysters required a longer exposure to higher temperatures. Populations with different responses were called "physiological races."

Hillman (1964) used paper chromatography to demonstrate differences in the patterns of free amino acids and small peptides between two populations of *Crassostrea virginica* from Long Island Sound and the James River, Virginia. Populational differences were also found by Li *et al.* (1967). Through the use of serological techniques, they found antigenic differences between two populations from the east coast of Canada.

Newkirk *et al.* (1977) found that larvae of parents from a low salinity population were more tolerant to low salinity than larvae of parents from a medium salinity area. Using crosses and progeny testing, they concluded that a cytoplasmic as well as a genetic factor was affecting the salinity tolerance of larvae from these populations.

Recently, in an electrophoretic study of the genera *Crassostrea* and *Saccostrea*, Buroker *et al.* (1979) included two collections of *C. virginica* from Nova Scotia and west Florida. These populations had low genetic similarities but intraspecific populations of other oyster species are nearly identical genetically. They suggest that the differences between these populations justify their designation as separate subspecies. The study also demonstrated that the west Florida collection and a collection of *C. rhizophorae* from the Virgin Islands were very similar, indicating that these forms are closely related.

Macrogeographic variation has been demonstrated electrophoretically in *Mytilus edulis* by Koehn *et al.* (1976). They analyzed 6 loci in 150 samples taken along the Atlantic Coast. At the LAP locus the most common allele ($p = 0.55$-0.59) was uniform from Virginia to Cape Cod, where there was a sharp decrease in frequency to 0.10 which continued north. A similar decrease occurred inside Long Island Sound. The GPI (PGI) locus exhibited correlation between allele frequencies and the latitude from which samples were taken. The other loci were homogeneous across the ranges.

In the present study, little genetic variation was indicated among the four northern populations. The Laguna Madre oysters, however, are genetically different. The data are consistent with results obtained by Dr. Wyatt Anderson (personal communication). He has analyzed oyster populations from along the Gulf of Mexico and Atlantic coasts. His results show that the Laguna Madre collections differ from other Gulf collections.

The large genetic differences between the Laguna Madre collection and the others may have risen from differential selection based on environmental differences or from isolation and genetic drift of alleles. The Laguna Madre population is both the most ecologically different and the most isolated population studied. Ecologically, the oysters in the Laguna Madre area are unusual in having adapted to hypersaline conditions (Bauer, 1962). Salinities in this area are higher than 35 ppt for several months each summer. Reefs of *Crassostrea virginica* rarely survive in salinities over 25 ppt.

The oysters of the lower Laguna Madre (near Port Isabel, Texas) are separated by a distance of 400 km from the nearest *Crassostrea virginica* populations in Redfish Bay, near Corpus Christi. No oysters are found in the Laguna Madre north of the Port Isabel area because of hypersalinity. Larval movement occurs by longshore currents along the Gulf side of Padre Island. Experiments by Smith (1975) and Watson & Behrens (1970) indicate that these wind-driven currents generally flow north in the summer but that net movement is low. During the winter there is often a convergence of two currents, one flowing south and one flowing north at the central portion of Padre Island. These studies suggest a low potential for migration between the lower Laguna Madre and oyster reefs to the north.

Comparison of Genetic and Morphological Differences

The pattern of genetic variation among collections is quite different from that of morphological variation. Morphologically, each population is distinguishable as indicated by shell measurements and other shell characteristics which cannot be easily quantified. The morphological variation is probably more closely related to environmental differences. It has long been known that the environment can

affect growth and shell characteristics of oysters. An experienced commercial oysterman can often determine the local origin of a catch of oysters merely by noting their shape, size, color and size of clusters. Factors which are known to affect these characteristics are salinity, current flow, turbidity and substrate (Galtsoff, 1964). Transplanted oysters can take on the characteristics of their new environment, which indicates a small genetic component to the morphological variation.

Of the five collections of oysters, the Laguna Madre collection looks the most different. These oysters had long, narrow, thin, crenulated, and highly colored shells. They formed larger clusters than usual. Cluster size and coloration could be used to distinguish the Laguna Madre and Aransas Bay collections even though the shapes of the oysters were not significantly different. The odd appearance of oysters from the Laguna Madre area was noted in several earlier papers (Hedgpeth, 1953 and Bauer, 1962). Parker (1955) reported evidence that the appearance is due to the environmental effect of hypersalinity. In a study of oyster growth in Aransas Bay, he found that oysters rapidly added new pigmented shell in response to high salinities which resulted from a two year drought. Periodic high salinity in Aransas Bay may explain the similarity in growth patterns of the Aransas Bay and Laguna Madre collections as indicated by the length/height index (Table 4).

Generally, this study shows that morphological and genetic variation are not related. The four northern populations were significantly different morphologically, but not genetically. The morphological variation is probably a response to differences in environmental factors such as salinity. The genetic variation does not seem to be related to these environmental differences.

SUMMARY

Oysters collected at five sites from Biloxi Bay, Mississippi to the Laguna Madre at the southern tip of Texas were analyzed for shell shape and five biochemical genetic markers. A statistical test showed that all of the samples were significantly different in morphology except the two most southern collections from the Laguna Madre and Aransas Bay, Texas. However, the statistical analysis of the biochemical genetic data demonstrates the genetic identity of all of the collections, except the one from the Laguna Madre. It is concluded that shell morphology is useful for determining environmental differences among oyster populations, but biochemical or genetic characters, or both, are necessary to study geographic differentiation in oysters.

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